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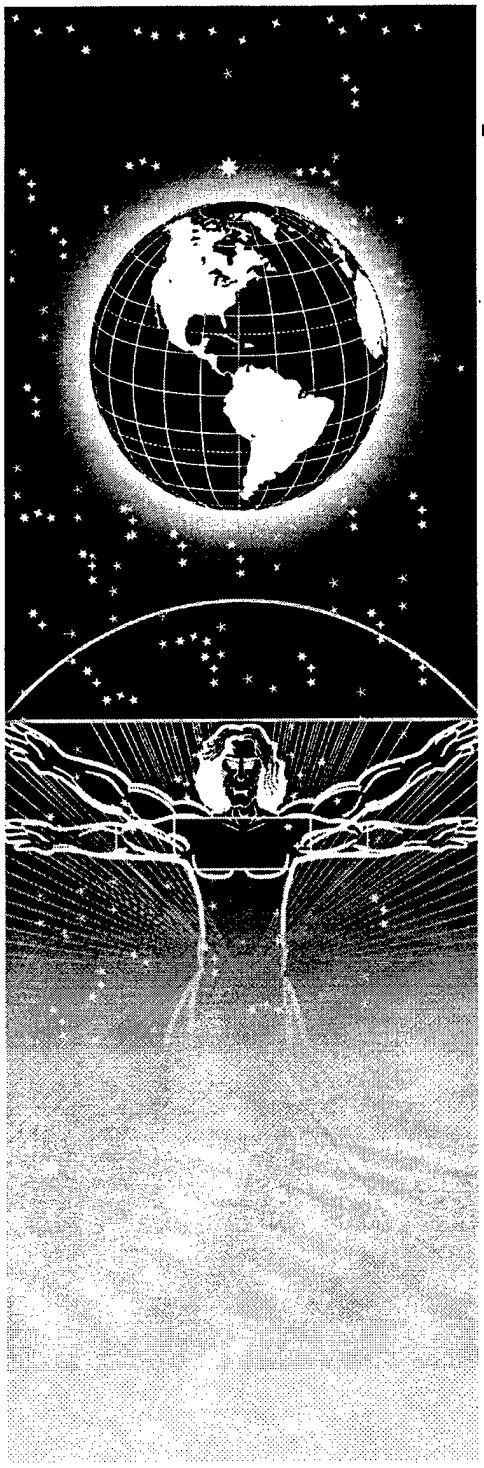

UNITED STATES AIR FORCE ARMSTRONG LABORATORY

Isolated Perfused Small Intestine-Application for Absorption and Metabolism of Trichloroethylene in the Fischer 344 Rat

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
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Exposure by ingestion is the major route of exposure to toxic substances, particularly chemicals found in groundwater. Toxic substances which enter the body by this route may be metabolized to toxic intermediates which may then be exposed in high concentrations to the liver through the "first pass effect", a physiological phenomenon occurring in the hepatic portal circulation. Because liver tumors in rodents are often the toxicity extrapolated to determine the "human risk", the intestinal absorption and metabolism of orally-administered compounds should be extensively characterized. The objective of this study was to develop and validate the isolated vascularly perfused rat intestine model to examine the intestinal metabolism and rate of uptake of Air Force relevant compounds. The test compound used in this study was trichloroethylene (TRI), a chlorinated aliphatic hydrocarbon which has been linked to the formation of hepatic tumors in mice and renal tumors in rats. This study was successful in demonstrating the ability of this technique to successfully perfuse the vasculature of the rat small intestine. Microscopic examination of intestinal tissue post-perfusion showed no signs of tissue edema or cell damage compared to control non-perfused tissue. An average cumulative uptake of approximately 0.04% of the administered dose was observed. In addition, observance of metabolite formation revealed small amounts of TCOH was produced. The isolated vascularly perfused small intestine model can be a powerful investigative tool applicable to a variety of biomedical disciplines. In the future, data derived from our application of this system will be used to refine our understanding of gut metabolism and absorbence of chemicals and their metabolites — processes vital to toxicants whose exposure is primarily oral.

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PREFACE

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

0_2Hb	Oxyhemoglobin
BSA	Bovine serum albumin
C	Celsius
cm	Centimeter
DCA	Dichloroacetic acid
et. al.	And others
GC-ECD	Gas Chromatograph(y) with electron capture detection
H_2O	Water
hr	Hour(s)
i.m.	Intramuscular
IU/mL	International units per milliliter
mg/kg	Milligrams per kilogram
mg/mL	Milligrams per milliliter
min	Minutes
mL	Milliliter
mm	Millimeter
nmol	Nanomoles
OSHA	Occupational Safety and Health Association
PE-50	Polyethylene-50
RBC	Red blood cells
TCA	Trichloroacetic acid
TCOH	Trichloroethanol
THb	Total hemoglobin
TRI	Trichloroethylene
uL	Microliter
US EPA	United States Environmental Protection Agency
x g	Multiplied by force of gravity

INTRODUCTION

Exposure by ingestion is a major route of exposure to toxic substances, particularly chemicals found in groundwater. The small intestine is a major site of absorption following an oral exposure, and the intestinal mucosa represents the first barrier through which an ingested xenobiotic compound must pass before entering the system. Toxic substances which enter the body by this route may exert toxicity locally within the gastrointestinal tract or at other sites following absorption. Therefore, an accurate description of the intestinal absorption of chemicals with a risk of oral exposure is essential to an accurate risk assessment prediction. In addition, chemicals may be metabolized to toxic intermediates or to non-reactive species by enzymes present in the lining of the GI tract or by endogenous microflora inhabiting the gut lumen. However, at present gastrointestinal metabolism has been only vaguely represented in pharmacokinetic models as "fraction absorbed". This metabolism has the potential to form a toxic intermediate which may then be exposed in high concentrations to the liver through the "first pass effect", a physiological phenomenon occurring in the hepatic portal circulation. Because liver tumors in rodents are often the toxicity extrapolated to determine "human risk", the intestinal absorption and metabolism of orally-administered compounds should be extensively characterized.

Information concerning the absorption and metabolism of toxicants by the gut will lead to understanding of how these compounds actually enter the systemic circulation and are distributed within the body. If a compound which is toxic until metabolized is absorbed into the systemic circulation at a rate higher than that which can be detoxified by the liver, a detrimental effect will be observed. Conversely, if the toxicant is shown to be metabolized by the small intestine, less of the toxic (parent) form of the chemical would be available to target organs and the observed effect would be milder than predicted from the absorbance of parent chemical.

The objective of this study is to develop and validate a procedure that will examine the intestinal metabolism and rate of uptake of Air Force relevant compounds. The test compound used in this study was trichloroethylene. Trichloroethylene (TRI) is a chlorinated aliphatic hydrocarbon which has been widely used as an industrial chemical since its synthesis at the beginning of the 20th century. Millions of workers are exposed to TRI each day [8,3] due to its use as a solvent for the vapor degreasing of metal parts, a solvent in the textile industry, and a lubricant, among other uses [5]. However, recent reports have linked exposure to TRI and its metabolites to the formation of hepatic tumors in mice and renal tumors in rats. For this reason, risk assessment of humans exposed to TRI is an increasing concern. In addition, due to its extensive use, TRI and its metabolites have been detected as soil and groundwater contaminants.

TRI has become a toxicant of acute interest to the Air Force due to the recent increase in military base closures. Environmental cleanup of base sites where these degreasing procedures were utilized has become an Air Force priority. For these reasons, a significant amount of effort has been dedicated to the complete characterization of TRI and its metabolites. Despite its high-profile status, one important aspect of the

characterization of TRI toxicity has previously been overlooked; the extent to which it is metabolized or absorbed by the small intestine.

Although no single experimental technique can provide all information about an intricate process such as absorption, the isolated perfused intestine technique offers many advantages to historical approaches [6] including maintenance of the isolated small intestine of a rat under conditions as close to physiological normality as practical and the description of the behavior of the intestine during perfusion. The isolated vascularly perfused rat intestine model also allows the precise *in situ* measurement of rates of uptake of toxicant and the capacity of the gut (and its contents) to metabolize compounds. More importantly, this procedure can achieve all of these effects while maintaining physiological blood pressure, blood flow and temperature. Our procedure was adapted from a technique described by Pang et al. [7] and Adams [1]. The advantages of this system are twofold: first, it will reveal the amount of compound actually available to target organs via direct measurement of absorption and metabolism in the small intestine. These findings will bridge the gap between exposure dose and absorbed dose. Second, the system will possess dual functionality: 1) to assess intestinal uptake and metabolism by injecting chemical into the lumen and sampling the perfusion medium over time; and 2) to measure intestinal extraction of chemical from blood by introducing the compound into the perfusion medium and plotting loss of chemical over time. In the future, data derived from our application of this system will be used to refine our understanding of gut metabolism and absorbance of chemicals and their metabolites--processes vital to toxicants whose exposure is primarily oral.

MATERIALS AND METHODS

Materials

Krebs-Ringer buffer was obtained from Sigma (St. Louis, MO) and prepared according to package insert. A 0.9% saline solution was prepared in the lab using sodium chloride from Sigma. Trichloroethylene was obtained from Aldrich (Milwaukee, WI). Heparin and bovine serum albumin were added to the Krebs-Ringer buffer and were supplied by SoloPak (Franklin Park, IL) and Pierce (Rockford, IL), respectively. Expired red blood cells were obtained from the Wright-Patterson Regional Medical Center. Experiments were conducted in closely controlled locations devoid of human traffic and proper blood and body fluid precautions were utilized. Medium containing red blood cells were disposed of via OSHA guidelines: medium was inactivated with sodium hypochlorite and disposed of as biological hazard for incineration.

Animals

Male Fischer-344 rats were obtained from Charles River Laboratories (Raleigh, NC) and were kept under quarantine for two weeks for histopathology examination. All animals were found to be in good health. Rats were housed in polycarbonate cages with hardwood chip bedding in a carefully controlled atmosphere of humidity and temperature. Purina lab chow (#5002, Ralston Purina) and *Pseudomonas*-free water was provided *ad libitum.* Lighting was on a 12 hour light/dark cycle beginning at 6:00 am.

Apparatus –

A Plexiglas box (49cm x 49cm x 39cm) was used to house most of the components used for warming and circulating the perfusate. Box temperature was maintained at 37°C with Thermolyne Heating Tapes (Barnstead/Thermolyne, Dubuque, IA), and air was circulated with a small fan mounted at the rear of the box (Archer, Radio Shack/Tandy, Ft. Worth, TX). A Harvard '66' Microprocessor Peristaltic Pump (Harvard Apparatus, Inc., South Natick, MA) circulated the perfusate from the reservoir via polyethylene tubing, through a 5mL plastic syringe which functioned as a bubble trap to prevent air embolism, and into the cannulated superior mesenteric artery of the rat. The cannula was constructed from a blunted 23 gauge needle fitted into a 3-4 cm length of PE-50 (Intramedic^R Clay Adams, Parsippany NJ) silastic tubing(ID 0.58mm OD 0.965mm). An 18 gauge polyethylene catheter (Angiocath^R Becton Dickinson Sandy, Utah) was inserted into the hepatic portal vein for collection of perfusate.

Preparation of Perfusate

The entire preparation was conducted on ice. Packed human red blood cells (RBCs) were removed from the bag via transfer line to a 50 mL culture tube. RBCs were then washed twice in 4 volumes of Krebs-Ringer buffer. A 10 minute centrifugation at 970 x g yielded an RBC pellet and a clear to pink-tinged supernatant. (A red supernatant indicative of hemolysis would have resulted in discarding the preparation). The pellet was resuspended to 20% in a solution of filtered Krebs-Ringer bicarbonate buffer prepared with 7 mg/mL BSA and 5 IU/mL heparin. In this procedure we were able to utilize RBCs which had been maintained (for training) up to 5 weeks past their expiration date.

Determination of Oxygenation

Oxygen content of the perfusate was determined with an IL282 CO-Oximeter. The IL282 CO-Oximeter is an automated instrument which measures total hemoglobin (g/dL THb), percent oxyhemoglobin (%O_2Hb), percent carboxyhemoglobin (% COHb), percent methemoglobin (%MetHb) and oxygen content (Vol % O_2) in whole blood.

A whole blood sample and three replicates of human packed red blood cells resuspended to 20% were individually aspirated into the instrument, each sample was mixed with diluent, hemolyzed, and brought to a constant temperature in the cuvette. Monochromatic light at four specific wavelengths passes through the cuvette to a

photodetector, whose output is used to generate absorbances. A dedicated microcomputer calculates the g/dL THb, $\%O_2Hb$, %COHb, %MetHb and Vol $\%O_2$ values for the samples [11].

Method

Our method is based upon previously described procedures [7,1]. Briefly, the rat was anesthetized with an i.m. injection of 70 mg/kg ketamine, 30 mg/kg xylazine. When sufficiently sedated, the rat was placed under a heating lamp connected to a YSI model 72 proportional temperature controller with probe (Yellow Springs Instruments, Yellow Springs OH) and the temperature was set at 37.2°C. Midline and lateral abdominal incisions exposed abdominal organs and vasculature. The intestines were gently exteriorized and surrounded with warm, saline-soaked gauze. Saline was employed throughout the procedure to keep all exposed tissues moist. The pyloric vein and bile duct were ligated. Loose ties were placed around the portal vein and the superior mesenteric artery (2 ties each). The left renal artery and vein were ligated. A loose tie was placed around the right renal artery and vein. The chest was opened and ketamine was injected directly into the heart in order to slow and eventually stop blood flow. The superior mesenteric artery was cut with ocular scissors and cannulated. The cannula was secured by tightening the distal tie around the cannula and artery. A continuous gravity-induced flush of oxygenated and heparinized (5 IU/mL) Kreb's-Ringer bicarbonate solution was used to perfuse the tissue immediately upon insertion of the arterial cannula. An 18 gauge catheter was quickly inserted into the portal vein and secured by tightening the loose tie over the inserted catheter and vein. The tissue was perfused with the bicarbonate solution only briefly while ties were secured and ligations were completed around right renal artery and vein, and proximal side of superior mesenteric artery. A visual check of perfusion was accomplished by verifying the color change of tissue accompanying erythrocyte removal. When cannulas were secured and ligations were completed, perfusion was begun with oxygenated and warmed (37°C) solution of 20% washed human red blood cells in Kreb's-Ringer buffer containing 7 mg/mL bovine serum albumin and 5 IU/mL heparin. Flow rate and pressure were allowed to stabilize before initiating experiments.

To initiate experiments trichloroethylene in a corn oil vehicle was injected directly into the lumen of the small intestine. Samples were collected from the cannulated portal vein in 2.0 mL extraction vials and kept on ice until all samples had been collected. Several different concentrations of TRI were injected.

Validation of Tissue Structure Post-Perfusion

Sections of small intestine which had been perfused with Krebs-Ringer bicarbonate solution were removed for histological examination along with sections of non-perfused tissue. The tissue sections were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 microns, and stained with hematoxylin and eosin (H&E). Tissues were examined for vessel integrity, signs of edema or other tissue damage.

Extraction Technique

Initially, red blood cells within the collected perfusate samples were lysed by vortexing them 3:1 in distilled H_2O for 1 min at room temperature in a Haake-Buchler vortex. Samples were then extracted in 1mL of iso-octane for 5 min at ambient temperature. Next, the vials were centrifuged to sediment the blood cells and placed in a -80°C freezer to solidify the lipids. The solvent phase was then assayed for TRI content by GC-ECD.

In addition, some samples were quenched with 20% lead acetate immediately after collection. These samples were analyzed for the metabolites trichloroacetic acid (TCA), dichloroacetic acid (DCA) and trichloroethanol (TCOH).

Analytical Procedure

A Hewlett-Packard model 5890 series II gas chromatograph equipped with an electron capture detector was used to analyze the collected samples. For the determination of TRI uptake by the intestine, direct injections of extract were made onto a stainless steel SP1000 packed column (6ft x $^1/_8$ in) and analyzed vs. an external standard curve of TRI in iso-octane. Extraction efficiency for each run was determined by means of direct injection of known quantities of TRI extracted from perfusate. This extraction efficiency was employed in the quantification of TRI taken up by intestine.
The chromatography conditions are shown in Table 1.

Table 1. Chromatography Conditions for Determination of TRI Uptake

Parameter	Setting
Initial temperature	130°C
Run Length	6.00 min
Heating rate	Isothermal
Carrier gas	Argon/Methane
Flow rate	25 mL/min
Make-up gas	Argon/Methane
Injection port temperature	175°C
Detector temperature	225°C
Oven temperature	130°C

The samples for the analysis of TCA, DCA, were derivatized to methyl esters with the addition of dimethyl sulfate, extracted with hexane and analyzed by gas chromatography. TCOH was extracted and analyzed without derivatization. The method used 2,2-dichloropropionic acid as an internal standard. Samples were injected by a Tekmar headspace analyzer onto a Hewlett-Packard 5890 GC equipped with a Supelcowax 10 capillary column (25m x 0.53 mm) and an ECD detector. Integration was accomplished with a Perkin-Elmer Nelson Turbochrome 3 data analysis system [12].

RESULTS

Assay of the oxygen content of the perfusate used in this procedure was carried out via an IL-282 CO-Oximeter (Table 2). Human packed red blood cells resuspended to 20% as described were stored at 4°C for four (4) days with no attempt to oxygenate. Results show that oxyhemoglobin levels exceeded 96.5% No further oxygenation of the perfusate is required. The hematocrit of the prepared perfusate was verified by comparison of total hemoglobin data from whole blood samples and prepared samples. Whole blood contains approximately 46% red blood cells, therefore one would expect the THb for that sample to be just over twice the value obtained for the 20% solution, which was observed.

Table 2. Hemoglobin Data from CO-Oximeter (IL-282)

ID	THb g/dL	% O_2Hb
Replicate #		
1	6.5	101.7
2	6.5	99.3
3	6.1	102.1
Whole Blood	16.5	74.7
Control	13.6	96.5

To demonstrate that the procedure was indeed capable of perfusing the vasculature supplied by the superior mesenteric artery; the small intestine, large intestine and cecum, without rupturing vessels or perfusing surrounding tissue (edema), a simulated perfusion was conducted using India ink in place of the perfusion medium. The dark blue ink contrasted well with surrounding tissue, enabling easy detection of vessel rupture or perfusate leakage into surrounding tissue. The ink in this simulated perfusion appeared to remain within the vasculature of the small intestine, as none of the surrounding tissue was visibly stained. Photo micrographs of hematoxylin and eosin stained perfused and non-perfused small intestine revealed that the perfusion process did not damage tissue or vessel integrity (Figure 1). The vasculature in this case was perfused with a red blood cell-free medium containing only heparinized Krebs-ringer bicarbonate solution. The presence of a saggital section of a vessel containing red blood cells distinguishes the non-perfused tissue from the perfused tissue. There is no evidence of the characteristic "onion-skin" effect surrounding perfused blood vessels that would indicate tissue edema: the condition of the basal epithelium of perfused tissue mirrors that of control. Figure 1 also shows that the epithelial nuclei are undisturbed in the perfused tissue, demonstrating that cellular integrity has been maintained.

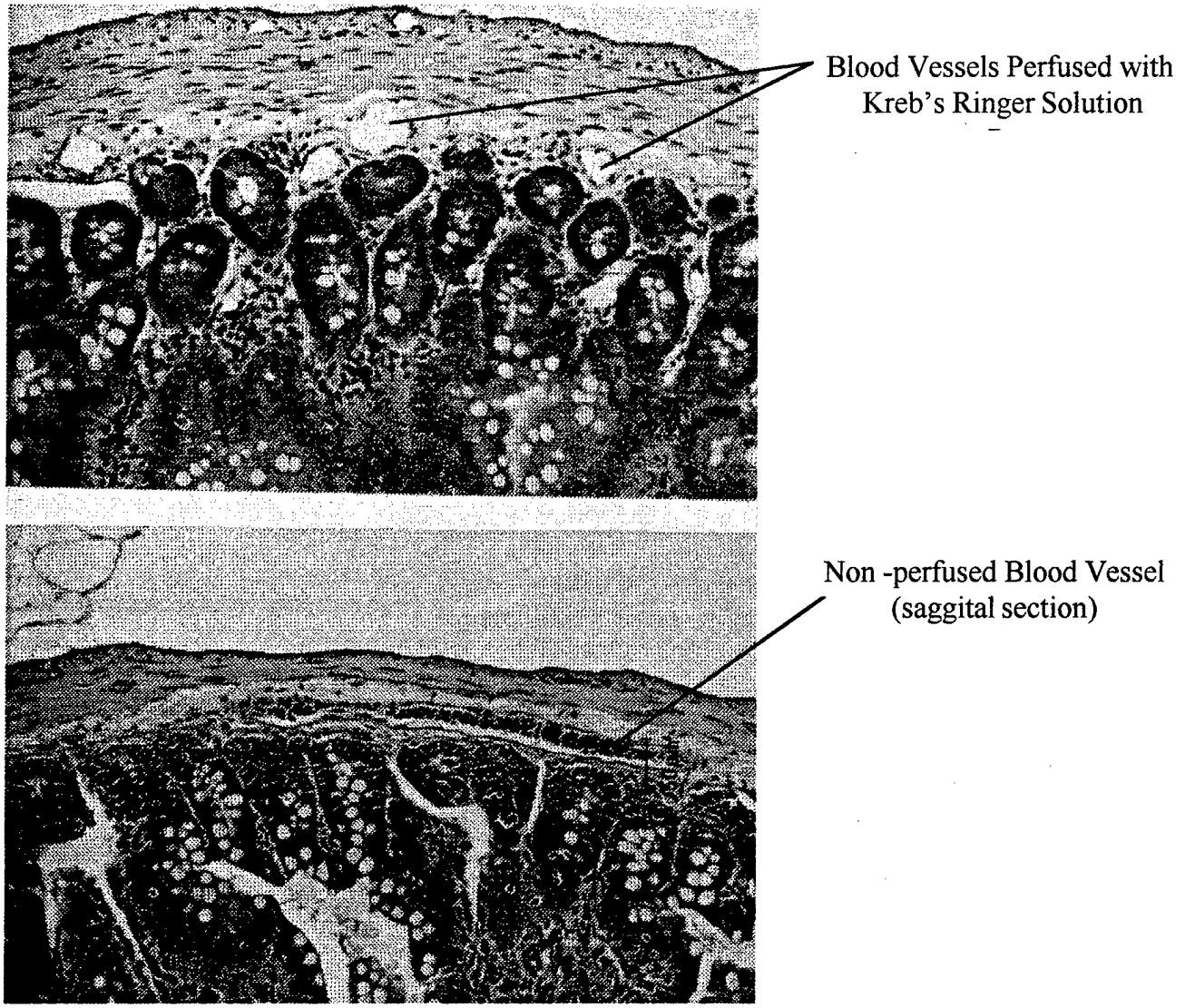

Figure 1. Microscopic view of small intestine of Fisher 344 rat. Top photo represents tissue perfused with the Kreb's Ringer Bicarb solution and illustrates that vessel integrity is maintained and edema is virtually absent. Bottom photo shows non-perfused intestine in which vessels still contain red blood cells.

The uptake of TRI by the small intestine was measured in five different perfusions. Rats were dosed at levels of 5, 25, and 50 mg/kg TRI in corn oil (Table 3).

Table 3. Dosage scheme for intestinal perfusions

Specimen #	Dose	Conc of Solution	Weight of Rat	Injection Vol.	mg TRI
1	5 mg/kg	5 mg/mL	240 g	0.24 mL	1.2 mg
2	5 mg/kg	5 mg/mL	240 g	0.24 mL	1.2 mg
3	25 mg/kg	25 mg/mL	210 g	0.21 mL	5.25 mg
4	50 mg/kg	50 mg/mL	220 g	0.22 mL	11.0 mg
5	50 mg/kg	50 mg/mL	250 g	0.25 mL	12.5 mg

Data was plotted as cumulative nmoles TRI taken up vs. time. Specimen #1 had a cumulative uptake of 0.737 nmoles TRI after 30 minutes of perfusion. This represents only 0.008% of the administered dose. Specimen #2, although subjected to the same amount of TRI initially, absorbed 2.952 nmol TRI after only an 11 minute perfusion, representing 0.032% of the administered dose (Figures 2,3). Partial explanation for this phenomenon can be found in the fact that the average flow rate for specimen #2 was 3.6 mL/min, while the average flow rate for #1 was only 0.67 mL/min

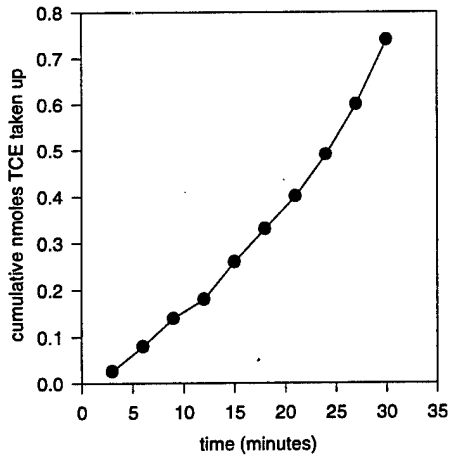

FIGURE 2 RAT INTESTINE PERFUSION--5 MG/KG TRI
30 minute perfusion performed on rat dosed with 5 mg/kg TRI in corn oil and perfused at an average flow rate of 0.67 mL/min.

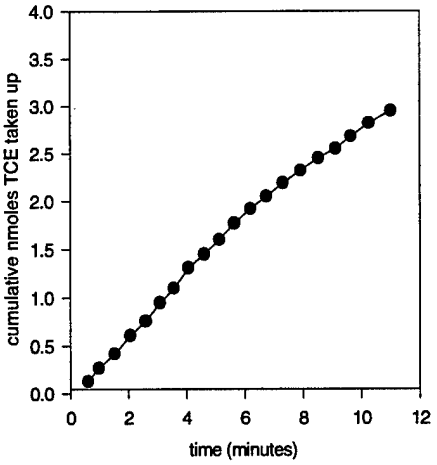

FIGURE 3 RAT INTESTINE PERFUSION-5 MG/KG TRI
11 minute perfusion performed on rat dosed with 5 mg/kg TRI in corn oil and perfused at an average flow rate of 3.6 mL/min.

Specimen #3 showed a cumulative uptake of TRI of 15.351 nmol, or 0.038% administered dose after 60 minutes (Figure 4). The perfusion lasted approximately one hour and had an average flow rate of 2.4 mL/min The average flow rate was determined

from measurements taken at the beginning and end of the run. The beginning flow rate was 3.7 mL/min but the rate fell to 1 mL/min by the end of the perfusion.

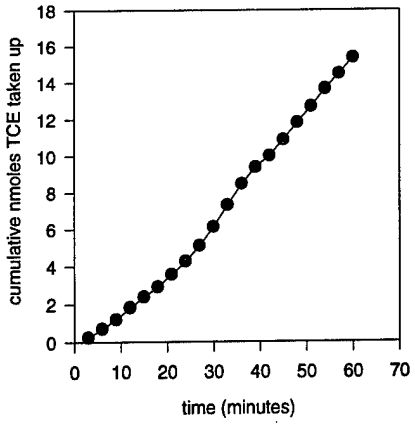

FIGURE 4. RAT INTESTINE PERFUSION--25 MG/KG TRI.
60 minute perfusion performed on rat dosed with 25 mg/kg TRI in corn oil and perfused at an average flow rate of 2.4 mL/min

Specimen #4 had a cumulative uptake of TRI of 10.335 nmol, or 0.012%, after 12 minutes of perfusion at 5.5 mL/min (Figure 5).When allowed to perfuse for a longer period of time (20 min) at the same rate as in specimen #5, the cumulative uptake of TRI rises to 88.974, which is 0.094% of administered dose (Figure 6). Although TRI uptake was not shown to level off in any of the perfusions, runs were nonetheless truncated due to limited supply of perfusate.

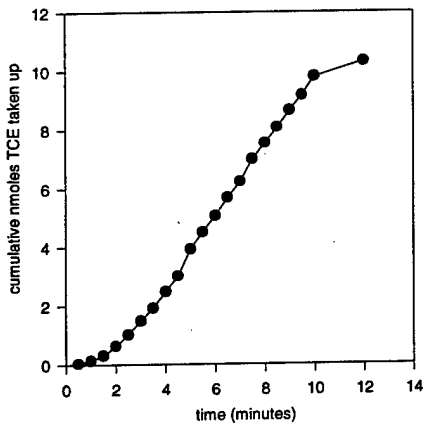

FIGURE 5. RAT INTESTINE PERFUSION--50 MG/KG TRI
12 minute perfusion performed on rat dosed with 50 mg/kg TRI in corn oil and perfused at a constant flow of 5.5 mL/min

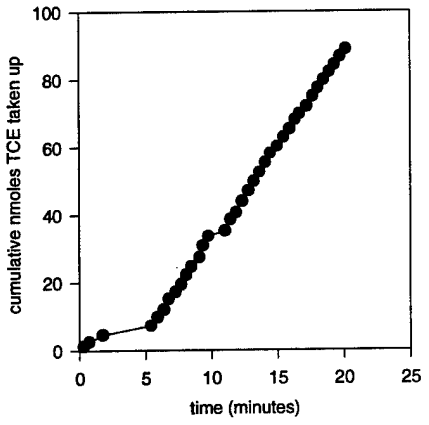

FIGURE 6. RAT INTESTINE PERFUSION--50 MG/KG TRI
20 minute perfusion performed on rat dosed with 50 mg/kg TRI in corn oil and perfused at a constant flow of 5.5 mL/min

Table 4 summarizes the five perfusions and compares the amount of TRI absorbed at 10 minutes at each administered dose.

Table 4. Comparison of TRI Uptake Across Doses

Specimen #	Dose	Duration of Perfusion	Flow Rate	nmol TRI Absorbed at 10 min	% of TRI Absorbed
1	5 mg/kg	30 min	0.67 mL/min	0.18	0.002
2	5 mg/kg	11 min	3.6 mL/min	2.95	0.032
3	25 mg/kg	60 min	2.4 mL/min	1.81	0.005
4	50 mg/kg	12 min	5.5 mL/min	9.80	0.012
5	50 mg/kg	20 min	5.5 mL/min	35.22	0.037

Metabolism of trichloroethylene does not appear to be occurring to a great extent in the small intestine of the rat. Samples collected from five perfusions were assayed for TCA, DCA, and TCOH content. In all five data sets chromatographic results showed that TCA and DCA was not produced in significant amounts. In two of the perfusions however, low levels of TCOH were formed (Figure 7). Results from both rats dosed at 5 mg/kg show the presence of low levels of TCOH in the collected perfusate. Data shows that at a flow rate of 0.67 mL/min TCOH levels increase linearly for approximately 18 minutes, then plateau at 2.6 cumulative nmol for the remainder of the run. When a flow of 3.6 mL/min was used TCOH levels were slightly higher and rise linearly for the entire 10 minutes as would be expected considering the previous data. At this rate, after 10 minutes of perfusing the intestine, 6.75 cumulative nmol TCOH was formed. TCOH levels were roughly proportional to perfusion flow (6.75 nmol TCOH/3.7 mL/min = 1.82; 1.37 nmol TCOH/0.67 mL/min = 2.04). These data may indicate that the uptake of TCOH is flow-limited.

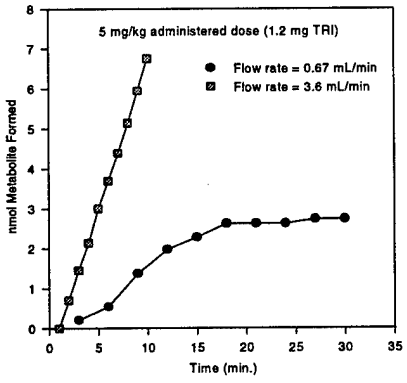

FIGURE 7. TCOH FORMATION FROM TRI IN RAT SMALL INTESTINE
Formation of trichloroethanol from intra-luminal injection of 5 mg/kg trichloroethylene in the proximal one-third of the small intestine of Fischer-344 rat.

It has been suggested that these results may be inconclusive due to the fact that samples indicating that TCOH is produced were not quenched with 20% lead acetate prior to analysis for presence of metabolites [9]. No TCOH was detected in any of the remaining perfusions which were all quenched with 20% lead acetate before analysis. It is possible that lead acetate may interfere with measurement of TCOH.

DISCUSSION

Trichloroethylene has been identified as a contaminant in several water supplies surveyed by the US EPA and is the contaminant most frequently identified. TRI is present in these contaminated supplies at concentrations in the low parts per billion, however it is likely that this toxicant is readily absorbed by humans [5]. The small intestine is the primary site of absorption and metabolism of orally administered toxicants yet, surprisingly, it has heretofore escaped scrutiny. The objective of this study was to introduce the isolated vascular perfusion technique as an investigative tool to assess the metabolism and absorption of trichloroethylene and other Air Force relevant toxicants by the small intestine. While this procedure has been criticized for being more elaborate than other *in vitro* techniques, it mimics actual physiological conditions much more closely. Variables such as temperature, pH, osmolality, blood pressure, flow rate, and perfusate composition can be easily controlled [6].

This study was successful in demonstrating the ability of this technique to successfully perfuse the vasculature of the rat small intestine as demonstrated by experiments with India Ink. Ink was used in place of the red blood cell media in this case to provide sharper visual contrast of perfused and non-perfused areas of the intestine. The ink was contained within the vessels of the mesenteric circulatory system but was not found in the surrounding tissue or adjacent vasculature, indicating that the vessels were not ruptured by the pressure exerted by the pump or by the perfusion procedure itself. Vessel integrity was further demonstrated by the photomicrographs. Microscopic examination of intestinal tissue post-perfusion showed no signs of tissue edema or cell damage compared to control non-perfused tissue (Figure 1).

Previous studies using this technique have shown that the intestine plays an important role in the metabolism of orally ingested toxicants. Hobara and coworkers found TRI to be readily absorbed from the small intestine of dogs. They demonstrated that the absorption rate in dogs was 50-70% of the administered dose after 2 hr. Further, they showed that no significant differences existed in the absorption rates of the different sections of the small intestine [5]. Hiriyama and Pang demonstrated the possibility of examining both the liver and the small intestine simultaneously in the metabolism of gentisamide and gentisamide conjugates. They postulated that the intestine, being anterior to the liver (with respect to an oral exposure route), would regulate the amount

and composition of substrates reaching the liver and therefore have a direct effect on the contribution of hepatic metabolism [4].

Contrary to previous findings concerning intestinal metabolism of trichloroethylene however, this study observed an average cumulative uptake of approximately 0.04% of the administered dose. In addition, observance of metabolite formation was limited to small amounts of TCOH, a result which has been questioned due to differences in sample quenching procedures.

Chemical characteristics which influence the rate and extent of absorption, such as lipophilicity of the test chemical, may be of concern in this procedure. Since TRI is a lipophilic substance, it has been suggested that the lymphatic system of the intestine might play a major role in the absorption of TRI [5]. In the intestine, lymphatic vessels function to absorb fat from the digestive tract and transport it back into the blood stream. However, the lymphatic system, unlike the cardiovascular system, has no mechanism to pump lymph back into the bloodstream. It must rely on the contraction of skeletal muscles which exerts pressure on the lymph vessels forcing lymph further along the vessel. In a sedated animal these muscle contractions are suppressed, therefore lymphatic flow can be expected to be reduced. Generally, lymphatic vessels which function as collecting vessels run alongside the arteries and veins of the cardiovascular system [10]. Special care should be taken not to rupture the lymphatic vessel that runs parallel to the superior mesenteric artery during cannulation. A ruptured vessel could interfere with normal lymphatic drainage, and perhaps alter the absorption of the chemical by the splanchnic blood supply. Due to overlooking the importance of maintaining an intact lymphatic system at the time of this study, particular care was not taken to ensure vessels were not disturbed. In some cases, absorption may have been altered due to damage to the lymphatic vessel parallel to the superior mesenteric artery.

The apparatus described in the Materials and Methods section of this paper is an updated version of the system used to gather the perfusion results presented in this paper. A more sensitive peristaltic pump, the Harvard '66', was added to achieve greater sensitivity and maintain a constant perfusate flow rate. Also, an Ashcroft 2500 series digital pressure monitor (Dresser Industries, Stratford CT) will be added before further data is gathered to ensure that physiological arterial pressure is maintained throughout the duration of the perfusion. With the implementation of the upgraded system and modification of the surgical procedure to ensure that the lymphatic system is not compromised, the isolated vascularly perfused small intestine model can be a powerful investigative tool applicable to a variety of biomedical disciplines.

Future experiments to further detail the validity of this system will be performed. Experimentation to address the issues surrounding the differences in results among species should be conducted before these data may be extrapolated to humans. Tissue viability studies involving glucose uptake and radiolabeled polyethylene glycol will further validate the techniques used and lend credibility to the results obtained. It may also be prudent to assay samples of collected perfusate for critical enzyme leakage as an indicator of tissue viability and integrity throughout the duration of the perfusion. The ability of the perfusate itself to metabolize TRI should be determined by spiking trichloroethylene directly into the perfusate and analyzing directly. Assessment of TRI uptake and TCOH formation with respect to flow rate should be examined and the impact

measurement of TCOH formation should be better characterized. Lastly the area of lymphatic contribution should be evaluated and the possibility of cannulating lymphatic vessels and collecting fluid directly should be determined.

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